

ORIGINAL ARTICLE

Pyruvic acid levels in serum and saliva: A new course for oral cancer screening?

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ABSTRACT

Objective: Cancerous cells show increased glycolysis rate. This will increase overall levels of pyruvate as it is one of the end products of glycolysis. The present on-going study is to estimate the levels of pyruvate in saliva and serum among healthy and oral cancer subjects. **Settings and Design:** Hospital-based cross-sectional comparative study. **Methodology:** A total of 50 subjects among healthy and oral cancer subjects were selected based on clinical and histological criteria. Saliva and serum samples were collected and subjected to pyruvate level estimation using biochemical analysis. **Statistical Analysis:** Descriptive analysis and Mann-Whitney test were used to find the statistical difference between the two independent groups. **Results:** Serum pyruvic acid levels of the healthy group were 1.09 ± 0.14 and for oral cancer, it was 2.95 ± 0.59 and salivary level were 3.49 ± 0.47 and 1.32 ± 0.10 respectively. Mann-Whitney test showed statistically significant difference in serum and salivary pyruvate level in between two groups ($P < 0.000$ respectively). **Conclusion:** The present study showed noticeable variation in the level of pyruvic acid among healthy and oral cancer subjects. This generates the hypothesis that estimation of the pyruvic acid can be a new tool to screening of the cancer.

Key words: Glycolysis, pyruvic acid, Warburg's effect

INTRODUCTION

Cancer is one of the major public health problems faced by most of the developed countries and is progressively increasing in the developing countries.^[1] According to the World Health Report 2004, cancer is the second most common cause of death in developed countries.^[1,2] It is known that cancer epidemic is due to the combined effect of the ageing of populations and the high or increasing levels of prevalence of cancer risk factors.^[1] It has been estimated that 43% of cancer deaths worldwide are due to tobacco, unhealthy diet, physical inactivity and infections.^[1,3] Oropharyngeal cancer is more common in developing than developed countries.^[3,4] In South-Central Asia, oral cancer falls under the three most common types of cancer and particularly in India, the age-standardized incidence rate of oral cancer is 12.6/100,000 population.^[1]

The U. S. Preventive Service Task Force in 1996 stated that available screening for oral cancer is limited to the physical examination of the mouth, a test of undermined sensitivity, specificity and positive predictive value. Approximately 70% of oral carcinomas are detected by visual inspection.^[5] At present, there are many cancer screening techniques including routine clinical examination, toluidine blue staining, tissue autofluorescence, oral brush biopsy and chemiluminescence.^[6] However, these methods have their own limitations such as acceptability, affordability and accessibility and inadequacy of training in oral cancer prevention and screening as self-assessed by physician, nurse practitioners and dental health professionals have been noted as important factors for possible delay in diagnosis.^[7]

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In spite of the tremendous progress in the field of molecular biology, there is yet no single marker that reliably enables to predict malignant transformation in an individual patient^[8] in more objectively. Hence, we are attempting a newer diagnostic method to predict the malignant transformation in oral cancer using serum and saliva as a medium. Pyruvic acid is an important intermediary cellular metabolic product of fat, protein and carbohydrates. It has been observed that cancer cells frequently disclose increase glycolysis and depend largely on this metabolic pathway for generation of adenosine triphosphate (ATP) to meet their energy requirements.^[9] However, whether the increase of glycolytic activity in cancer cells is mainly due to inherent metabolic alterations or due to the anaerobic environment in the tumor tissues remains controversial^[10,11] Potentially malignant disorders also showed a significant increase of pyruvic acid levels.^[12] Hence, there is a chance of imbalance in the production and excretion of the pyruvic acid in the local and systemic aspects of oral cancer subjects.

Estimating the level of pyruvic acid in saliva and serum might reflect the spectrum of oral cancer. Hence, the aim of the present study is to estimate and compare the levels of pyruvic acid in saliva and serum in normal and oral cancer subjects.

METHODOLOGY

Approval was sought from the Institutional Ethical Committee. Patients who visited the outpatient ward were enrolled. Data were obtained, by one student researchers and one academic researcher. A convenience sample was used with a total of 50 subjects.

Two groups, i.e., Healthy and oral cancer were selected based on confirmed clinical and histological reports. Subjects over 40 years of age and the individuals who gave informed consent were chosen for the study. The individuals who gave negative clinical report were enrolled in the healthy group; however, subjects with positive clinical and histological reports, regardless of grades and stages were recruited in the oral cancer group. Diagnosed oral cancer subjects were incorporated in the study. Elimination criteria included systemic infections such as cardiac diseases, diabetes and other carbohydrate metabolic disorders and subjects under chemotherapy, radiotherapy and surgery. Demographic information and data with respect to the personal habits, medication, past dental and medical history were collected from all the two groups.

The subjects selected for the study were asked to give 3 ml of unstimulated saliva and 5 ml of venous blood.

Unstimulated saliva collection

Patients were asked to press their tongue against palate so that freshly secreted saliva would be collected in the sublingual region. Three milliliters of collected saliva was allowed to drool into sterile plastic tubes.

Venous blood collection

Five milliliters of venous blood were collected from a median cubital vein in antecubital fossa and transferred to sterile plastic tubes coated with heparin. Blood was mixed with heparin by shaking the tubes gently.

Immediately saliva and blood samples were transferred to deep freeze to halt the rate of biochemical and bacterial responses and then transferred to the biochemical laboratory within 1 h of sample collection.

Biochemical procedure

Blood pyruvate quantification was done in the following steps:^[13]

- I. 2 ml of heparinized blood was mixed with 4 ml of 0.6M per chloric acid and kept in an ice bath for 10 min, then the mixture was subjected to centrifugation of 3000 rpm for 5 min and supernatant fluid was collected
- II. The 3 ml of supernatant fluid was mixed with 1 ml of di-potassium phosphate solution and this solution was centrifuged at 3000 rpm for 10 min and again the supernatant fluid was collected. This supernatant fluid obtained was protein free filtrate of blood sample
- III. 1 ml of dinitro phenyl hydrazine was added to each solution and kept at 37° centigrade for 10 min [Figure 1]
- IV. 10 ml of freshly prepared 0.4M sodium hydroxide was added and quantified with spectrophotometry (Shimadzu) of wavelength 540 nm after 10 min
- V. Blank and standard solutions of pyruvic acid were analyzed simultaneously in similar way.

Salivary pyruvate estimation

Step I and II were bypassed due to fewer amounts of protein levels in saliva when compared to serum^[14] and step III, IV, and V were performed in a similar way as blood pyruvic acid quantification.

On relating healthy with oral cancer subjects, Kolmogorov-Smirnova and Shapiro-Wilk test showed no normal distribution; thereby Mann-Whitney test was used to measure the statistically significant difference between the groups.

RESULTS

In total, 50 subjects were recruited. Of the participants 25 (13: males; 12: females) belonged to healthy group and 25 (18: males; 7: females) were included in oral cancer group. The mean age of the healthy group was 55.44 ± 9.56 and oral cancer group was 55.52 ± 11.07 [Table 1].

Comparison of pyruvic acid levels of healthy with oral cancer

Serum pyruvic acid levels of the healthy group were 1.09 ± 0.14 and for oral cancer, it was 2.95 ± 0.59 . Mann-Whitney test

showed statistically significant difference between the groups ($P < 0.00$).

Similarly in saliva, pyruvic acid levels were high in cancer subjects (3.49 ± 0.47) compared to healthy subjects (1.32 ± 0.10) and showed statistically significant difference between the groups ($P < 0.00$) [Table 2 and Figure 2].

DISCUSSION

In the physiologic process of glycolysis, pyruvic acid and lactic acids were produced as the end product.^[7] This yields 2 ATPs of energy from a single glucose molecule. This energy production cascade continues by utilizing the end product (pyruvate) of glycolysis into Krebs’s cycle in mitochondria by oxidative phosphorylation. Although, this method of production of ATPs is not seen in cancer cells,^[15] increasingly glycolytic pathway is used to maintain energy supply. Cancer cell shows changes not only in the glycolytic pathway but also in the Krebs cycle, beta-oxidation and anabolic metabolism. In general, they are reoriented to respond to the new primary function of the cell (i.e., uncontrolled proliferation) by providing not only energy but also the synthesis of nucleotides, amino and fatty acids.^[9,16-19]

Many factors influence the mitochondrial respiratory function.^[11] Some of the carcinogens induce mutation of mitochondrial DNA, malfunction of the electron transport chain, aberrant expression of enzymes involved in energy metabolism and insufficient oxygen as available in the cellular microenvironment. This leads to impaired production and functioning of 13 important protein components of mitochondrial respiratory complexes.^[11]

Hypoxia also can initiate the Warburg’s effect as the rate of angiogenesis is comparatively less than that of the rate of growth of the tumor, this will induce depleted oxygen environment in the neoplastic area and induces alternative respiratory method for the energy production and its survival,^[11] i.e., glycolysis pathway. Hence, glycolysis is a central metabolic pathway that

finely regulates cell proliferation by adapting the cancer cell’s metabolism to the conditions of its current selective situation.^[20-24]

Cancer cell exhibits increased glycolysis rate for their energy need which produces more of its end products such as pyruvic acid and lactic acid. At room temperature, lactic acid converts into pyruvic acid as it is chemically unstable. This phenomenon increases the overall level of pyruvic acid in the body. This excess pyruvic acid either leaches into the blood or oral cavity. Therefore, quantification of pyruvic acid might give the proportional level of severity of oral cancer. Hence, we used quantification of pyruvic acid for this study.

The serum pyruvic acid estimation was performed by the hydrazine method of Lu and Friedemann-Haugen. However, as it estimates not only pyruvic acid but also other keto-acids, it delivers false negative results in the study.^[25] Hence, Landon and his colleagues have shown a modified way of estimating the pyruvic acid from serum.^[26] The mean serum pyruvate

Table 1: Distribution of subjects in gender and age

Subjects	n	Mean age±SD
Healthy	25	55.44±9.56
Males	13	56.46±9.62
Females	12	54.33±9.8
Oral cancer	25	55.52±11.07
Males	18	54.11±12.06
Females	7	59.14±7.51

SD: Standard deviation

Table 2: Mann-Whitney U-test on healthy and oral cancer group

Groups	Medium	Mean±SD	Mean rank	P (two-tailed)
Healthy	Serum	1.09±0.13	13.88	0.000*
	Saliva	1.32±0.10	13.00	
Oral cancer	Serum	1.78±0.42	37.12	
	Saliva	2.52±0.41	38.00	

*Significant association set at ≤ 0.05 . SD: Standard deviation



Figure 1: Biochemical method of pyruvic acid estimation

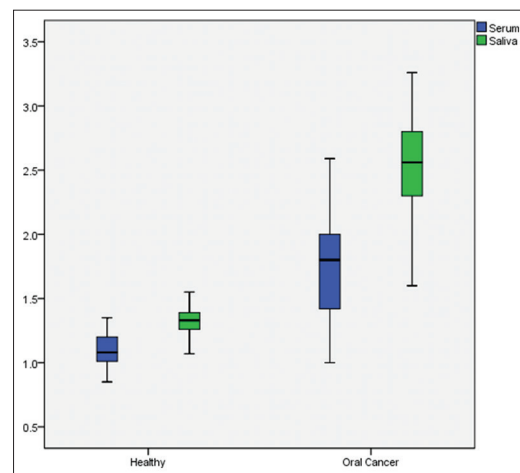


Figure 2: Distribution of pyruvic acid level in serum and saliva among healthy and oral

level from our results is 1.09 ± 0.13 mg which is similar to the level estimated using a hydrazone method.^[18]

Salivary pyruvic acid level for healthy individuals was found to be 1.46 ± 0.64 ,^[12] which is in parallel with this study (1.32 ± 0.10). This is slightly higher than the serum levels, one of the reasons may be the presence of oral bacteria. The prokaryotic bacteria, devoid of mitochondria,^[27] uses only glycolysis pathway for their energy requirement and survival.

The present study result showed elevated Pyruvic acid level in saliva than in serum; this may be because the serum pyruvic acid levels get balanced with the process of Cori's cycle^[28] occurring in the liver and another reason might be due to leaching out of pyruvic acid into oral cavity locally.

The external validity of this study depends on further evaluation of the results in a different setting like multi-center trials involving cross population analysis. One of our limitation is the internal validity which depends on adequate sample size based on population prevalence of oral cancer.

CONCLUSION

The present ongoing study with a limited number of samples showed that there is a noticeable variation in the level of pyruvic acid among healthy and oral cancer subjects. This generates the hypothesis that estimation of pyruvic acid can be a stepping stone for the screening of cancer. Further studies in this direction will provide clearer vision to this field.

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Conflicts of interest

There are no conflicts of interest.

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